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Greenhouse gas emissions from the energy crop oilseed rape (*Brassica napus*); the role of photosynthetically active radiation in diurnal N₂O flux variation

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Abstract

Oilseed rape (OSR, *Brassica napus* L.) is an important feedstock for biodiesel; hence, carbon dioxide (CO₂), methane (CH₄) and particularly fertilizer-derived nitrous oxide (N₂O) emissions during cultivation must be quantified to assess putative greenhouse gas (GHG) savings, thus creating an urgent and increasing need for such data. Substrates of nitrification [ammonium (NH₄)] and denitrification [nitrate (NO₃)], the predominant N₂O production pathways, were supplied separately and in combination to OSR in a UK field trial aiming to: (i) produce an accurate GHG budget of fertilizer application; (ii) characterize short- to medium-term variation in GHG fluxes; (iii) establish the processes driving N₂O emission. Three treatments were applied twice, 1 week apart: ammonium nitrate fertilizer (NH₄NO₃, 69 kg-N ha⁻¹) mimicking the farm management, ammonium chloride (NH₄Cl, 34.4 kg-N ha⁻¹) and sodium nitrate (NaNO₃, 34.6 kg-N ha⁻¹). We deployed SkyLine2D for the very first time, a novel automated chamber system to measure CO₂, CH₄ and N₂O fluxes at unprecedented high temporal and spatial resolution from OSR. During 3 weeks following the fertilizer application, CH₄ fluxes were negligible, but all treatments were a net sink for CO₂ (ca. 100 g CO₂ m⁻²). Cumulative N₂O emissions (ca. 120 g CO₂-eq m⁻²) from NH₄NO₃ were significantly greater ($P < 0.04$) than from NaNO₃ (ca. 80 g CO₂-eq m⁻²), but did not differ from NH₄Cl (ca. 100 g CO₂-eq m⁻²) and reduced the carbon sink of photosynthesis so that OSR was a net GHG source in the fertilizer treatment. Diurnal variation in N₂O emissions, peaking in the afternoon, was more strongly associated with photosynthetically active radiation (PAR) than temperature. This suggests that the supply of carbon (C) from photosynthate may have been the key driver of the observed diurnal pattern in N₂O emission and thus should be considered in future process-based models of GHG emissions.

Keywords: automatic chamber, biodiesel, carbon dioxide, denitrification, greenhouse gas, methane, nitrification, nitrogen, nitrous oxide, oilseed rape

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Introduction

Carbon dioxide (CO₂) has risen from pre-industrial levels of 280 ppm (IPCC, 2007) to around 410 ppm and is widely acknowledged to be driving anthropogenic climate change (IPCC, 2014; Carlton *et al.*, 2015). Other biogenic greenhouse gases (GHGs), nitrous oxide (N₂O) and methane (CH₄), having global warming potentials over 100 years (GWP) of 298 and 34 times that of CO₂ (Myhre *et al.*, 2013), have also increased from pre-industrial levels by more than 50 and 250%, respectively

(Conrad, 2009; Myhre *et al.*, 2013). As a consequence, some of the most sensible and emerging strategies for reducing national greenhouse gas (GHG) burdens specifically tackle these more potent GHG gases. However, before mitigation strategies can be implemented, a concerted effort to reduce the huge uncertainty ($\pm 37\%$) in estimates of N₂O emissions (Committee on Climate Change, 2017) is needed.

Generally, during growth, crops in Europe sequester carbon (C) from the atmosphere (Schulze *et al.*, 2010), and European agricultural land, are also a net sink for CH₄ (Ciais *et al.*, 2010). In contrast, one of the biggest global sources of N₂O is agriculture (Reay *et al.*, 2012) and, in 2013, agricultural N₂O contributed approximately 8%

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of the UK's annual net GHG emissions, more than half of the emissions from transport and all industrial emissions (DECC, 2015). Accounting for more than 30 Mt CO₂ equivalents per year, N₂O is the single biggest contributor to UK agricultural GHG emissions (DEFRA, 2014a), and arable farming, as a result of the application of fertilizers, is an especially large emitter of N₂O.

Oilseed rape (OSR, *Brassica napus* L.) was grown on 36 million ha in 2014 (FAO 2017), 6.5 million ha of which are found in continental Europe, a greater area than used for potatoes, sugar beet, pulses and even maize (ec.europa.eu; http://ec.europa.eu/eurostat/statistics-explained/index.php/Main_annual_crop_statistics). In the United Kingdom, 11% of available agricultural land (675 000 ha) was dedicated to its cultivation in 2013 (DEFRA, 2014b) and it is typically grown in rotation with wheat (*Triticum aestivum* L.) or barley (*Hordeum vulgare* L.). Whereas, in the United Kingdom, rapeseed oil is used mainly for food products, in Europe, OSR is the most widely used feedstock for biodiesel (De Vries *et al.*, 2014), where 6 Mt (ca. 60%) of rape oil is used for this purpose (AHDB 2017a). As GHG mitigation is a key aim of using OSR for energy production, it is essential that accurate accounting of all its associated GHG emissions is prepared to assess the putative GHG savings. This requirement will be particularly exigent when the EU's Renewable Energy Directive, setting a 50% GHG reduction target for biofuels compared to fossil fuels, comes into action in 2018 (EU, 2009), whilst the default GHG saving from OSR is just 38% (Gerasimchuk, 2013). This shortfall might be expected to reduce the demand for OSR diesel, but 2016 saw record volumes produced, and industry analysts predict that whilst the OSR biodiesel fraction of total biofuel production must drop, the absolute volume required will remain unchanged as the total output of bioenergy production in the EU must increase to meet the 2020 target of 10% (AHDB 2017b).

Measurements from soil under OSR have shown considerable variation in the magnitude of N₂O fluxes, ranging from <40 µg m⁻² h⁻¹ (Barton *et al.*, 2010) to over 2000 µg m⁻² h⁻¹ (Drewer *et al.*, 2012). Studies of GHG fluxes from OSR (Hellebrand *et al.*, 2003; Barton *et al.*, 2010; Drewer *et al.*, 2012; Asgedom *et al.*, 2014) have generally relied on manual chambers, deployed with sampling frequencies of once a month up to a maximum of five times a week, focussed around fertilization events. Due to the size of OSR, chambers rarely include the vegetation, but where they do (Jeuffroy *et al.*, 2013), the use of opaque chambers dictates that reported CO₂ fluxes are ecosystem respiration and not net ecosystem exchange (NEE); with the exception of a single study in Germany (Kutsch *et al.*, 2010), there is an alarming scarcity of NEE data for this important crop.

The scarcity and low temporal resolution of appropriate data hinder our understanding of the magnitude of GHG source–sink dynamics and the driving processes associated with OSR.

Knowledge of the controlling processes of GHG fluxes facilitates design of GHG mitigation strategies, and whilst the processes controlling ecosystem CO₂ (Reay & Grace, 2007) and CH₄ (Le Mer & Roger, 2001) fluxes are well understood, those controlling N₂O fluxes are less clear. Of several microbial N₂O production pathways, nitrification and denitrification are considered the most important in soils (Smith, 2017). The former is the aerobic oxidization of ammonium (NH₄⁺) to nitrate (NO₃⁻), whilst the latter is an anaerobic sequence of heterotrophic reactions through which NO₃⁻ is reduced to dinitrogen gas (N₂) via N₂O and requires a carbon (C) source (Wrage *et al.*, 2001). Nitrogen fertilizer is applied in many forms; as soils differ in their capacity for nitrification or denitrification (Bateman & Baggs, 2005), fertilizer type can affect consequential N₂O fluxes (Dobbie & Smith, 2003a; Zhang *et al.*, 2014; Zhou *et al.*, 2014). Ultimately, both nitrification and denitrification depend on nitrogen (N) substrate availability (Dalal *et al.*, 2003), but multiple pathways and other contributing factors, soil temperature, moisture, pH (nitrification) (Parton *et al.*, 1996), soil organic carbon availability (denitrification), oxygen (O₂) concentration, water-filled pore space (WFPS) (Davidson *et al.*, 1993) and soil respiration (Castaldi, 2000) (denitrification) ensure that N₂O fluxes are notoriously difficult to predict, especially at fine temporal resolution (Fitton *et al.*, 2014b).

Despite this lack of understanding of variation in N₂O emissions, rudimentary management guidelines already exist regarding the timing of fertilizer application (Environment Agency, 2015). These are designed to prevent N losses during rain through leaching and N₂O emissions but could benefit markedly from a fuller understanding of the processes governing N₂O fluxes to reduce future emissions (Rees *et al.*, 2013). Currently, IPCC tier 1 emissions factors (EF) guidance states that ca. 1% of applied N will be lost as N₂O over the course of the following year (De Klein *et al.*, 2006), but the accuracy of this method has been called into question, particularly for Europe (Gerber *et al.*, 2016).

Oilseed rape typically receives between 100 and 200 kg N ha⁻¹ in fertilizer over the course of its cultivation (DEFRA, 2010); therefore, understanding the response of OSR to N fertilization and developing the ability to reduce N₂O emissions from this crop would constitute a substantial saving in the UK's agricultural GHG footprint. In natural ecosystems, given the appropriate combination of conditions, as much as 20% of the total annual N₂O flux may be emitted in just 48 h (Mummey *et al.*, 1997). In agricultural systems, N₂O

emissions have been seen to increase rapidly in the weeks following N fertilizer (Ambus *et al.*, 2010), sometimes by two or three orders of magnitude (e.g. Dobbie & Smith, 2003b; Liu *et al.*, 2005), and emissions have also been shown to vary up to 200% on a diurnal scale (Shurpali *et al.*, 2016). As both sources and sinks of this trace gas fluxes can exist within a landscape, fluxes can be spatially and temporally heterogeneous (Chadwick *et al.*, 2014; Kravchenko & Robertson, 2015). Without continuous measurements of N₂O flux at an appropriate spatial resolution, the potential for failure in detecting significant emission events persists.

Eddy covariance (EC) can measure landscape scale GHG fluxes at high frequency, but cannot resolve measurements to the smaller plot scale. This lack of fine spatial resolution severely hinders the ability of an investigator to conduct replicated manipulation experiments, which are vital for advancing understanding of the mechanistic controls of net GHG flux and validating mitigation strategies. In contrast, chambers are ideal for measuring at the small spatial scale, but the frequency of data produced using manual chambers is limited by the availability of personnel, with the associated laboratory analysis of gas samples being both time-consuming and unsuitable for real-time monitoring. Automation, whilst expensive, increases the frequency of measurements, but chambers are frequently opaque to prevent overheating and are usually too small to accommodate any vegetation taller than a few centimetres. We deployed a novel automated system (SkyLine2D) incorporating a single, transparent, mobile chamber, suspended from an aerial rope transect, enabling reliable repeated near-continuous measurement of GHG fluxes from predesignated measurement positions. By circulating the chamber headspace through a series of analysers, the system was capable of delivering a full GHG budget for CO₂, CH₄ and N₂O from an intact OSR crop at relatively low cost.

The objectives of this study were to provide an accurate GHG budget from OSR following fertilizer application, to characterize the short- to medium-term variation in GHG fluxes and to establish the processes driving N₂O production from OSR following application of N fertilizer. Three mineral N treatments [ammonium nitrate (NH₄NO₃), ammonium chloride (NH₄Cl) and sodium nitrate (NaNO₃)] were applied to test the hypothesis that GHG fluxes would significantly differ depending upon the form of N applied to the crop.

Materials and methods

Study site

The study was conducted on a 7-ha field which was part of a working farm in the east of the United Kingdom. The field had

been drilled with OSR in November 2013, and inorganic fertilizer was applied three times between 1 March and 1 April 2013. The field had been planted with barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) in rotation, and the crop immediately preceding the OSR had been spring barley. The soil type was the Beccles 1 association (Drewer *et al.*, 2012) with fine silt over clay, and the field was used to produce annual rotation arable crops. Bulk density at the site was measured as $1.33 \pm 0.20 \text{ g cm}^{-3}$ (0–10 cm depth) and $1.49 \pm 0.14 \text{ g cm}^{-3}$ (10–20 cm depth).

Experimental design

All measurements presented were made between 24 March and 14 April 2014 since this is the period of fastest crop growth and hence the time the farmer applied fertilizer. During the study, the crop height increased from <10 cm to nearly 1 m; the main flower buds were present but closed by 31 March (GS5.4), began to open by 2 April (GS6.0), and the crop was in full flower by 13 April (GS6.5). Prior to this study, the first N fertilizer application to the crop ($67.5 \text{ kg N ha}^{-1}$) occurred on 5 March, with two subsequent mineral N applications of $68.9 \text{ kg N ha}^{-1}$ during the experiment on 24 March and 1 April. Background N₂O fluxes were measured on 18 March from the experimental transect and shown to be $144 \pm 50 \mu\text{g m}^{-2} \text{ h}^{-1}$. The experimental applications mimicked the NH₄NO₃ fertilizer ('FER') treatment on five replicate plots (within 40-cm-diameter collars), with additional ammonium-only ('NH₄') as NH₄Cl and nitrate-only ('NO₃'), as NaNO₃ treatments. The treatments were applied in pellet (NH₄NO₃) or powder form to each collar on a *pro rata* basis so that FER collars received the same N dose ($68.9 \text{ kg-N ha}^{-1}$) as the rest of the field, whilst the NH₄ and NO₃ treatments received the equivalent dose as the respective component parts of the fertilizer (i.e. NH₄: $34.6 \text{ kg-N ha}^{-1}$; NO₃: $34.4 \text{ kg-N ha}^{-1}$). Care was taken to ensure the treatments were applied evenly within the area of the collars, to mimic the action of the spreader. Nitrogen additions were applied within one hour of the farmer's fertilizer application to the field, during which time the measurement collars were covered with plastic sheeting to avoid any stray inputs within the experimental collars.

Greenhouse gas flux measurements

The SkyLine2D automated chamber system was developed in-house at the University of York. A single, cylindrical chamber was suspended from a motorized trolley, mounted on parallel horizontal ropes, 1 m apart and held above the crop by 2.5-m-tall aluminium trellis arches (Fig. 1), placed 24 m apart, allowing a trolley to repeatedly traverse a preselected transect across the crop. An indexing system identified designated 'stops' at which the chamber automatically lowered to conduct a measurement. Each landing base (collar) for the chamber consisted of a flat, horizontal circular flange of expanded polyvinyl chloride (PVC) with an inner diameter of 38 cm (Fig. 2) with a perpendicular PVC collar which was inserted ca. 2 cm below the soil surface to achieve a seal. Upon completion of the programmed measurement period at a collar, the chamber

automatically lifted and the trolley moved to the next 'stop'. The sequence in which collars were sampled was programmable, allowing for randomization or exclusion of specific collars, if required. In addition to automated operation, the system could be controlled manually, allowing an operator to move the trolley between points and drop and raise the chamber, as necessary.

The SkyLine2D chamber was cylindrical and made of clear Perspex and a size (internal diameter = 40.74 cm, height = 62 cm, volume = 80 820 cm³, Fig. 2) designed to completely accommodate the mature OSR crop over which the GHG flux measurements were made. Attention had to be given to ensuring that the growing crop was cleanly enclosed within the dropping chamber as the crop heightened, and this was achieved using loose stringing of the crop within the footprint of the base ring as it grew. The chamber was designed as a nonsteady state dynamic chamber, with headspace gas being circulated from the chamber through analytical equipment and returned through an umbilical via polyethylene tubing (Bev-A-Line IV, Cole-Parmer, London UK; internal diameter 3 mm, length 7 m). The aperture for the sampling tube was situated 10 cm from the top of the chamber (approximately 60 cm above the soil surface) and the gas return tube entered 5 cm above the bottom lip of the chamber (Fig. 2), avoiding sampling from directly above the soil

surface, yet assisting in the mixing of the headspace gas. The base of the chamber was fitted with an ethylene propylene diene monomer (EPDM) rubber seal (Top Bubble Gasket, part no. 490750, Essentra Components, Milton Keynes UK) which formed a gas-tight closure when dropped on the flange of the landing base (Fig. 2), with a pressure sensor inside the seal being activated when the chamber was fully closed. Guides around the chamber bases ensured the chamber landed accurately, and to minimize pressure differences associated with closing a chamber over the soil, a vent was incorporated into the design of the chamber, after Xu *et al.* (2006). The system included a safety feature which would halt operation at high wind speeds; this threshold could be adjusted and was determined empirically through observation of the system's performance.

Greenhouse gas flux analysis

A Licor infrared gas analyser (IRGA: LI-8100; Licor, Lincoln NE USA) was housed in the motorized trolley to measure CO₂ concentrations and also to control the SkyLine2D chamber, acting in place of a Licor long-term automated chamber (LI-8100-101; Licor). The Licor software was used to calculate linear CO₂ fluxes, adjusted for temperature, chamber volume

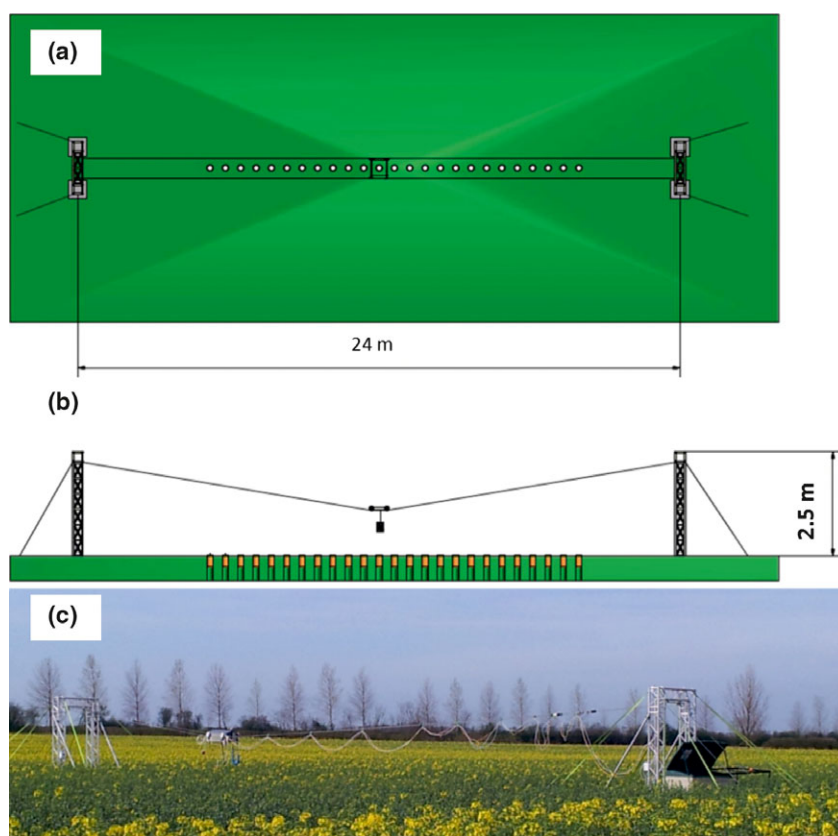


Fig. 1 Aerial and side profile schematics of the SkyLine2D system showing (a), the trellis arch supports at either end, supporting the Kevlar ropes between. The motorized trolley is depicted at the mid-point of the two supports (b). Cross section of the *in situ* system at the OSR field site and (c) the N₂O and CH₄ Los Gatos CRD analysers were housed in the green garden box by the right-hand trellis support. CRD, cavity ring-down.

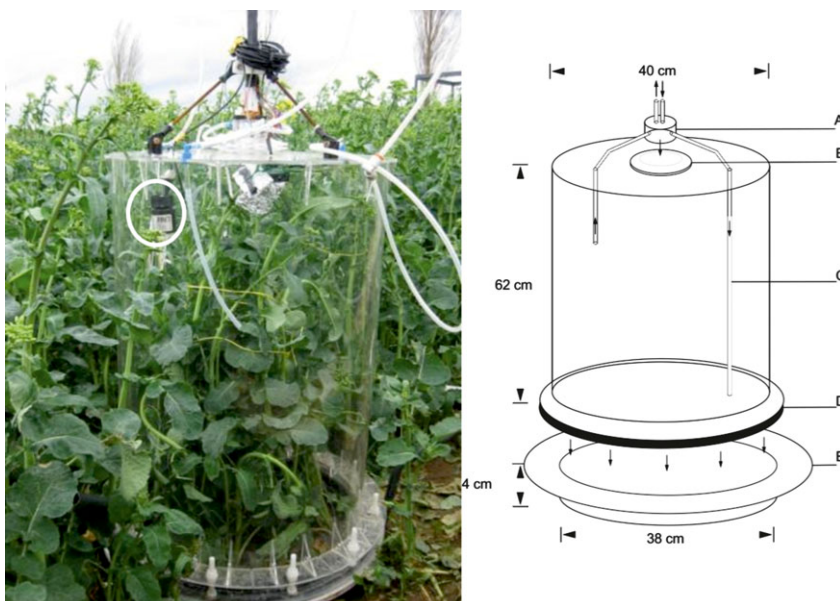


Fig. 2 The SkyLine2D chamber *in situ* during a measurement over the OSR crop (left-hand panel). Note the PAR sensor mounted within the chamber (white circle). The schematic of the chamber (right-hand panel) highlights the components and dimensions: (a) manifold with attached gas lines. Arrows denote direction of flow; the sampling line draws from near the top (circa 10 cm) of the chamber, and the return pipe opens near the base of the chamber. (b) Vent for pressure equalization, after Xu *et al.* (2006). (c) Chamber constructed from clear Perspex. (d) Gasket to ensure gas-tight seal between chamber and e- landing base. The base (ring) had an inner diameter (38 cm) smaller than that of the chamber (41 cm), affording a greater margin of error when the chamber landed. OSR, oilseed rape; PAR, photosynthetically active radiation.

and enclosed soil area, following Healy *et al.* (1996). In order to also measure the fluxes of N₂O and CH₄, the exhaust from the IRGA was intercepted through T-pieces and fed via an additional 49.8 m of Bev-A-Line tubing to separate cavity ring-down (CRD) laser analysers for N₂O and CH₄ flux measurements (LGR isotopic N₂O analyser and LGR fast greenhouse gas analyser, Los Gatos Research, CA, USA) housed in an enclosed shed at one end of the SkyLine2D apparatus (Fig. 1). The gas for analysis was circulated in series, the stronger flow rate of the internal pump of the CH₄ analyser dictated that it was placed first in the sequence and a shunt for any overpressure was used to compensate for different flow rates, before returning to the chamber. Both CRD analysers measured at 1 Hz, and fluxes were calculated as the change in concentration over time using linear regression, with a correction for volume, temperature and soil area. Chamber closures of 10 min were programmed for the flux measurements, with a gap of 5 min between chamber closures to allow refreshing of the chamber with ambient air. For each closure, a 60-s 'dead band' was allowed for head-space mixing, then a two-minute period was used for the regression to calculate CO₂ flux and a four-minute period used for N₂O and CH₄ fluxes. Following this protocol, each cycle (the term used to designate a full series of measurements across the transect) was 270 min long, allowing for approximately six measurements at each of the 18 sampling points per day. The attenuation of light by the chamber was calculated by linear regression from concurrent measurements of photosynthetically active radiation (PAR) inside and

outside of the chamber using two matched PAR sensors (SKP 215; Skye Instruments, Powys, Wales, UK) attached to a data logger (GP1; Delta-T Instruments, Cambridge UK), measuring at 1 Hz over the 21 days of the study period; this revealed a reduction of 29% in PAR inside the chamber. After determining the extent of light interception, CO₂ flux measurements were further adjusted during hours of daylight (defined as periods where external PAR > 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$) using the equation from a light-response curve, as described by Heinemeyer *et al.* (2013).

Ancillary measurements

High-frequency (1 min, averaged over 15 min) measurements of soil moisture and temperature at 5 cm depth were made in the centre of each landing base using temperature (UA-001-64 HOBOWare; Onset Corporation, MA, USA) and moisture probes (S-SMDM005; Decagon Devices Inc, WA, USA).

Statistical analyses

All statistical analyses were conducted using SAS (SAS, 9.4; SAS Institute, NC, USA). Quality control of flux calculations was initially performed by discarding faulty chamber closures and then using the output statistics from the linear regression of each chamber closure: if the R² value of the CO₂ flux was below 0.9, fluxes were discarded; for N₂O and CH₄ fluxes, nonsignificant ($P > 0.05$) regressions were then counted as zero fluxes.

Cumulative fluxes were calculated by trapezoidal integration, but due to a series of power failures, after 6 April, flux measurements tended to be intermittent so the cumulative fluxes of all three GHGs are calculated here only up to that date.

Where GHG flux data were not normally distributed, N₂O flux rates were log transformed and the reciprocal of the CO₂ fluxes were used. For repeated measures analysis, a mixed effects model was used to study the effects of time and N treatment on GHG fluxes (collar and block as random factors), pairwise comparisons were made using least squares, accounting for multiple comparisons using Tukey's range test. Two-way analysis of variance was carried out on cumulative N₂O fluxes to test for effect of N treatment and sampling hour, and treatment effect was also tested on cumulative GHG balance using analysis of variance; post hoc testing was undertaken using Duncan's multiple range test. Due to the large variation in absolute fluxes over the study, in order to investigate diurnal patterns, fluxes of both CO₂ and N₂O were normalized, achieved using the highest daily value of flux to constrain the data (forcing all normalized flux values to fall between 0 and 1). The total N₂O-N emitted over the study was calculated as a percentage of the total mineral N applied in the two experimental applications (24 March and 1 April) to give an estimate of the emission factor.

Results

The SkyLine2D system performed well producing ca. 4 000 flux measurements of the three major biogenic GHGs; CO₂, N₂O and CH₄ over the study. The equipment worked equally well both day and night, and air temperatures within the chamber never differed from ambient by more than 5°C over a full 10-min chamber closure; 95% of measurements were within 3°C of ambient, and using only the first three minutes of the closure for NEE measurements, the effect of any temperature increases was minimized.

GHG response to nitrogen fertilizer treatment

All flux measurements of N₂O showed a net emission from the soil to the atmosphere (by convention referred to here as a positive flux). Initial fluxes (24–30 March), three weeks after the initial pre-experimental fertilizer application, were very low and did not exceed 250 $\mu\text{g m}^{-2} \text{h}^{-1}$ during this period (Fig. 3a). Four days after the first NH₄NO₃ ('FER'), NH₄ only ('NH₄') and NO₃ only ('NO₃') fertilizer additions on 27 March, fluxes began to increase and, during the afternoon of 29 March N₂O emissions from all treatments were close to 500 $\mu\text{g m}^{-2} \text{h}^{-1}$, a rate which was maintained until the second N addition on 1 April. By the second N addition, fluxes were approaching 1000 $\mu\text{g m}^{-2} \text{h}^{-1}$ (Fig. 3a) with distinct peaks in N₂O emission during the afternoons of 31 March to 6 April. These peaks increased steadily from ca. 500 $\mu\text{g m}^{-2} \text{h}^{-1}$ on the 31 March to a

maximum of 3131 $\mu\text{g m}^{-2} \text{h}^{-1}$ on the 6 April and the highest mean flux (4266 $\mu\text{g m}^{-2} \text{h}^{-1}$) was recorded from the NH₄ collars on 6 April, with a further peak in N₂O emissions from all treatments seen on 12 April.

There was a significant effect of the N treatments on N₂O emissions, $F_{[2,356]} = 9.76$, $P < 0.0001$, and there was a significant interaction between treatment and time over the study, $F_{[122,356]} = 1.35$, $P < 0.02$; during the 16 h following the first application of the three N treatments, emissions from the NO₃ collars were significantly higher than from either the NH₄ or FER plots ($P < 0.05$). During the period 4–11 days after the N applications (between 28 March and 5 April), fluxes were greatest from the FER treatment; over several cycles, N₂O fluxes were significantly higher ($P < 0.04$) than at least one of either the NO₃ or the NH₄ treatments and for three cycles were higher than both the other treatments. No further statistically significant pairwise treatment effects were observed after this point, although the NH₄ plots tended to be highest during the peak following the second N addition.

Net ecosystem exchange of CO₂ (NEE) was characterized by positive fluxes (net emission) during hours of darkness, when respiration was the dominant process, and negative fluxes (net uptake) during the daytime when the OSR was photosynthesizing. The amplitude of the oscillation between positive and negative fluxes increased through the study period as the crop grew and flowered which coincided with a rise in soil and air temperatures. Highest CO₂ emissions (ecosystem respiration) were seen overnight on 30–31 March (700 $\text{mg m}^{-2} \text{h}^{-1}$) and 5–6 April (898 $\text{mg m}^{-2} \text{h}^{-1}$) (Fig. 3b), and these peaks followed the two dates that showed the greatest net uptake in CO₂ (maxima of −1953 and −1765 $\text{mg m}^{-2} \text{h}^{-1}$, respectively). N treatments did not have a significant effect on NEE throughout the study, $F_{[2,574]} = 1.38$, $P > 0.29$.

There was also no significant effect of the N treatments on CH₄ fluxes ($F_{[2,398]} = 0.15$, $P > 0.86$) (Fig. 3c), and whilst fluxes were often negative, indicating the soil was a net sink for CH₄, all net fluxes were close to zero, with a mean, maximum and minimum of 3, 150 and −140 $\mu\text{g m}^{-2} \text{h}^{-1}$.

Diurnal GHG flux patterns

In addition to the diurnal pattern of NEE, throughout the study, a clear and repeating diurnal trend in N₂O emissions was also observed, with peaks in the afternoon and lows throughout the night (Fig. 4). Analysis of this diurnal variation in N₂O fluxes (and to a lesser extent NEE) was confounded during periods where dramatic changes in flux rates occurred (two orders of magnitude in as little as three days for N₂O).

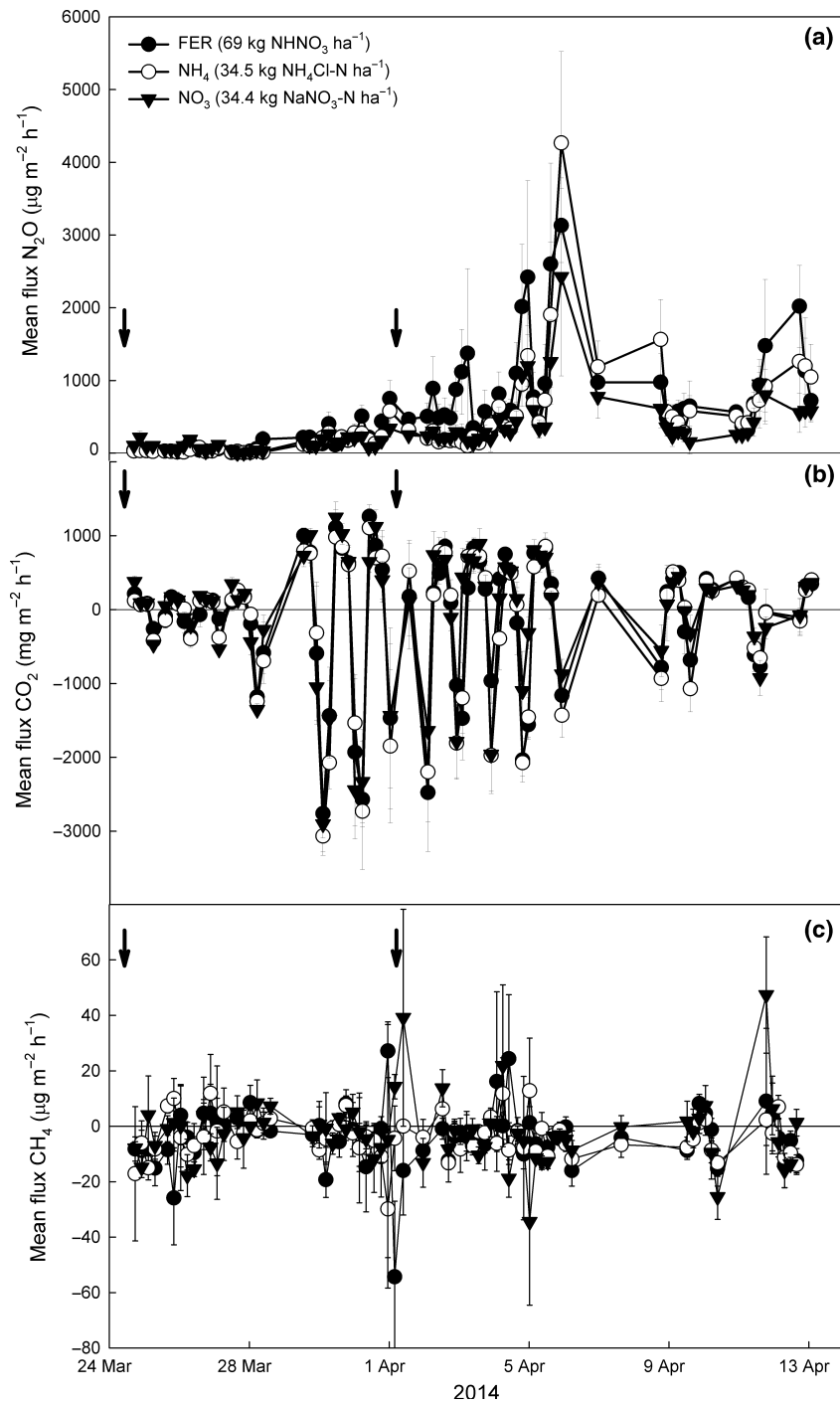


Fig. 3 Fluxes of N₂O (a), CO₂ (b) and CH₄ (c) from the oilseed rape crop, following application of three types of mineral nitrogen (NH₄NO₃ (FER), closed circles, NH₄Cl (NH₄), open circles, NaNO₃ (NO₃), closed triangles) measured using the SkyLine2D. Values shown are mean ($n = 5$, $\pm 1SE$). Vertical arrows indicate timing of nitrogen additions.

Normalizing the flux data showed that the maximum N₂O emission consistently occurred during the afternoon, peaking around 13:00 for the FER treatment, 14:00 for NH₄ and around 12:00 for the NO₃ treatment (Fig. 5a) which coincided with maximum net ecosystem

production (NEP) (greatest net uptake of CO₂) for all three nitrogen treatments (Fig. 5b). This characteristic is further reinforced by the strong positive relationship between the normalized fluxes of N₂O and CO₂ for each N treatment ($P < 0.0001$; Fig. 6).

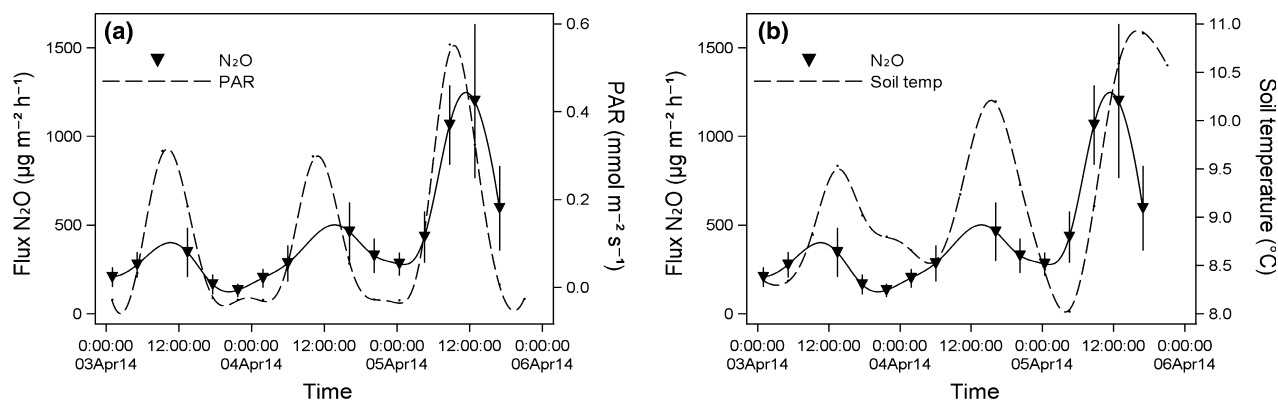


Fig. 4 Diurnal variation of N_2O flux in relation to photosynthetically active radiation (PAR) (a) and soil temperature at 5 cm depth (b). Data shown are for the collars treated with NaNO_3 (NO_3). Fluxes of N_2O can be seen to increase prior to soil temperature and in close relations to PAR.

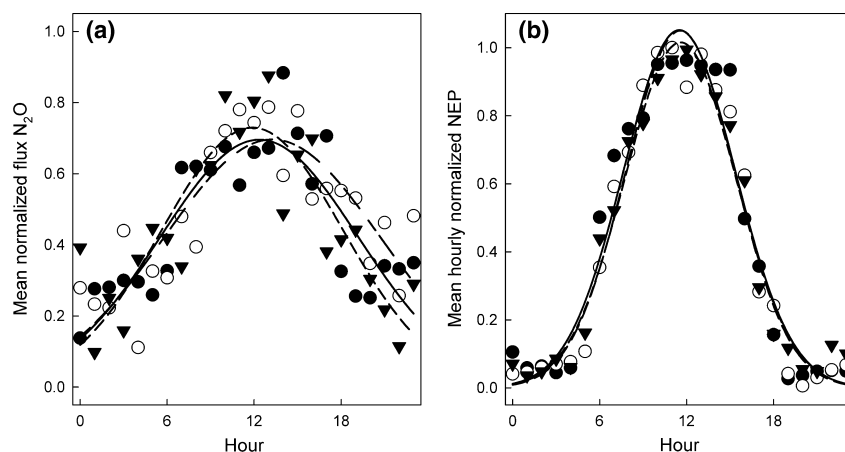


Fig. 5 Diurnal variation of the mean ($n = 5$) daily normalized N_2O (a) and NEP (b) averaged over the entire study period. Data are shown for each of the three nitrogen treatments applied, and a third-order Gaussian function has been fitted: FER – closed circles, solid line: N_2O $R^2 = 0.74$, $P < 0.0001$; NEE $R^2 = 0.94$, $P < 0.0001$; NH_4 – open circles, long dashes: N_2O $R^2 = 0.70$, $P < 0.0001$; net ecosystem exchange (NEE) $R^2 = 0.97$, $P < 0.0001$; NO_3 – closed triangles, short dashes: N_2O $R^2 = 0.75$, $P < 0.0001$; NEE $R^2 = 0.97$, $P < 0.0001$.

Environmental controls on GHG fluxes

When the absolute fluxes (non-normalized) were analysed across all dates, the strongest correlation between N_2O fluxes for the FER and NH_4 treatments was with soil temperature (Fig. 7a) whilst PAR also correlated with N_2O fluxes in the NO_3 treatment (Fig. 7b), although none explained more than 35% of the variance of these fluxes. These analyses did not explain the key driver of the diurnal variation in N_2O flux, and when the normalized fluxes were correlated with the measured environmental variables, it was clear that PAR had the strongest relationship with both NEE, in a typical light-response relationship similar across all three N treatments (Fig. 7c) and strikingly with N_2O emissions as well, again across all three N treatments ($R^2 > 0.62$; Fig. 7d).

Cumulative fluxes and GHG balance

The strong diurnal pattern in N_2O flux raises concerns about the choice of sampling time used to estimate cumulative fluxes for N_2O . As not every collar was measured hourly on each day, fluxes were binned into six 4-h subperiods revealing a strong significant effect of sampling time on the cumulative N_2O flux ($F_{[5,72]} = 8.05$, $P < 0.0001$); measurements taken between 12:00 and 16:00 yielding a greater total emission estimate than at any other time of day (Fig. 8). The cumulative flux was significantly lower from NO_3 collars than from the FER treatment ($F_{[2,72]} = 3.62$, $P < 0.04$, Fig. 8), and whilst there was no significant interaction of sampling time and treatment ($F_{[2,72]} = 0.64$, $P > 0.77$), the difference between estimates based on 09:00–12:00 and

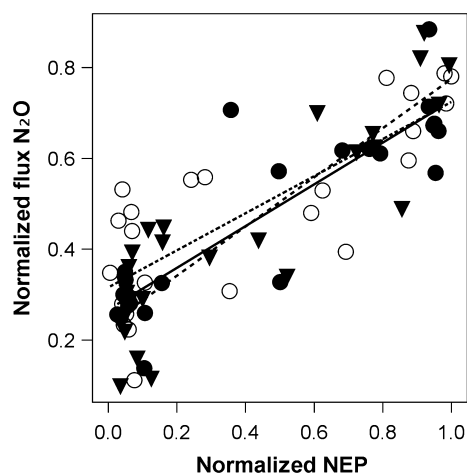


Fig. 6 Relationship of the mean hourly normalized flux of N₂O to the mean hourly normalized flux CO₂ (expressed as net ecosystem production (NEP)) across the study period. Data shown are for three nitrogen treatments: FER – closed circles, solid line: $R^2 = 0.77$ $P < 0.0001$; NH₄ – open circles, long dashes: $R^2 = 0.64$ $P < 0.0001$; NO₃ – closed triangles, short dashes: $R^2 = 0.75$, $P < 0.0001$.

12:00–16:00 were less pronounced for the NO₃ treatment than for the other two treatments. These fluxes represented a total loss over 14 days of FER 1.06 (± 0.23), NH₄ 0.86 (± 0.23) and NO₃ 0.64 (± 0.21) kg N₂O-N ha⁻¹ which equated to 0.77, 1.25 and 0.92%, respectively, of the total N applied during the study period.

The OSR field was a net sink for CO₂ from 24 March to 6 April, accumulating FER 107.5 (± 23.5), NH₄ 170.4 (± 16.94) and NO₃ 115.1 (± 16.0) g CO₂ m⁻², with no significant effect of N treatment ($F_{[2,12]} = 2.24$, $P < 0.15$, Fig. 9). The contribution of CH₄ to the overall balance was negligible at <0.3% of the total GHG balance across all treatments, but due to the magnitude of N₂O emissions, the GHG sink was greatly reduced in the NO₃ and NH₄ treatments and the FER treatment was identified as a net weak source of GHGs (Fig. 9). The overall GHG balance did not significantly differ between N treatments ($F_{[2,12]} = 2.85$, $P < 0.1$).

Discussion

In contrast to the clear response of N₂O flux to fertilizer, no effect was apparent in NEE, and CH₄ fluxes were so small and their contribution to the GHG balance was negligible. The increase in NEE between 28 and 30 March coincided with an increase in both PAR and air temperature, and the similarity of NEE and biomass between nitrogen (N) treatments (unpublished data), despite FER receiving twice the N of the other treatments, indicated growth was not N limited. Maximum NEE reported here was similar to a controlled

environment study of OSR (Paul *et al.*, 1990), but below that of a field trial conducted under higher light and temperature conditions (Muller *et al.*, 2005). N₂O fluxes were similar to the short-term response to N fertilizer Drewer *et al.* (2012) reported, but were between three (Hellebrand *et al.*, 2003; Kavdir *et al.*, 2008; Asgedom *et al.*, 2014) and ten times greater than reported elsewhere (Beaudette *et al.*, 2010) for similar rates of mineral N application to OSR. With the exception of Drewer *et al.* (2012), who measured N₂O flux in the hours immediately following fertilization, these studies employed a weekly to monthly measurement regime, suggesting that low temporal resolution is a major factor in the lower fluxes reported therein.

Cumulative N₂O flux (equivalent to 0.77–1.25% of applied N across the three treatments) counteracted most, and in the FER treatment all, of the sink effect of photosynthesis over the study. These values are not strictly emission factors, as an untreated control was not required to test our hypotheses, and this should be considered when interpreting these cumulative emissions. Despite this, the amount of N emitted as N₂O over just 14 days of our study fell within the IPCC inventory annual estimates of fertilizer emissions (De Klein *et al.*, 2006); thus, the final total may be above those guidelines. As OSR is the principal feedstock for biodiesel in Europe (De Vries *et al.*, 2014), it is essential that accurate measurements of N₂O fluxes are included in any lifecycle analysis (LCA), especially as a net GHG source was seen in the FER treatment (NH₄NO₃) reflecting the regimen employed by the farmer. The magnitude of GHG emissions due to high N input further supports existing scepticism (Smeets *et al.*, 2009; Del Grosso *et al.*, 2014; Walter *et al.*, 2015) regarding the effectiveness of OSR as an energy crop.

Not all field studies measuring agricultural N₂O fluxes at an appropriate temporal frequency report diurnal patterns (e.g. Barton *et al.*, 2008; Lognoul *et al.*, 2017), but several have shown N₂O emissions peaking during the afternoon (e.g. Ryden *et al.*, 1978; Blackmer *et al.*, 1982; Christensen, 1983; Livesley *et al.*, 2008; Simek *et al.*, 2010; Alves *et al.*, 2012; Das *et al.*, 2012; Marsden *et al.*, 2017), attributing this to soil temperature patterns (Blackmer *et al.*, 1982; Livesley *et al.*, 2008; Alves *et al.*, 2012). The daytime peak may be as much as 200% of night-time emissions (Shurpali *et al.*, 2016) which isotopologue data indicated to be due to increased denitrification (Ostrom *et al.*, 2010). Dissolved CO₂ in tree xylem can contribute to measured NEE (Levy *et al.*, 1999), and N₂O has also been measured from tree leaves (Pihlatie *et al.*, 2005). Calculations based upon maximum measured transpiration in OSR, ca. 8 g m⁻² h⁻¹ (Pivec *et al.*, 2011), and the solubility of N₂O at 15°C (5.95×10^{-4} mol mol⁻¹), suggest that, whilst

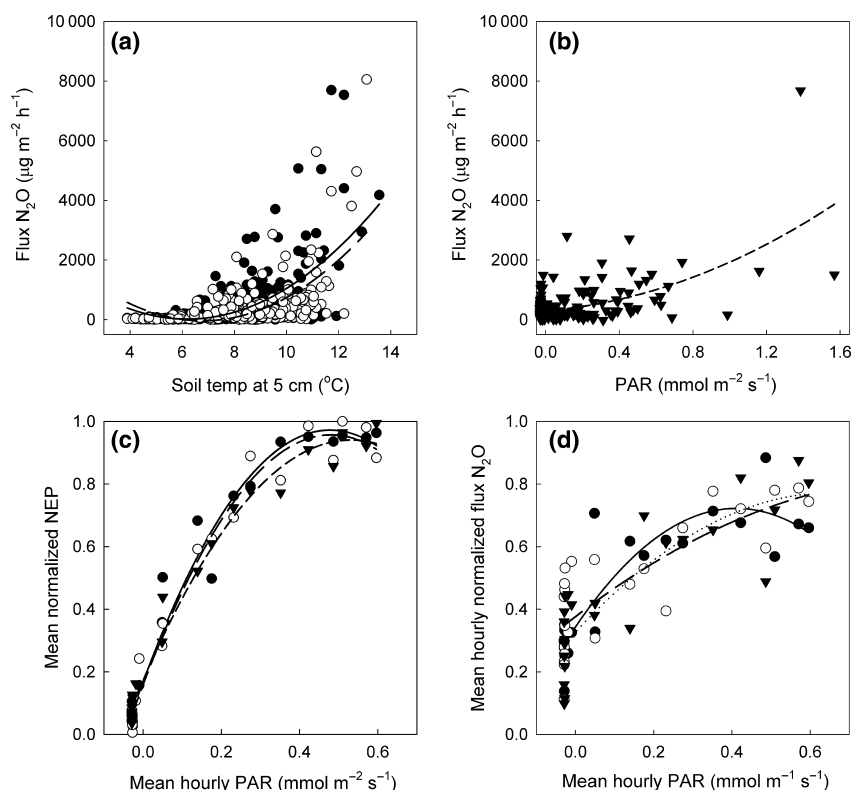


Fig. 7 Response of N₂O flux from oilseed rape (OSR) to soil temperature at 5 cm depth (a) under two nitrogen treatments: FER – (closed circles, solid line) $R^2 = 0.35$, $P < 0.0001$; NH₄ – (open circles, long dashed line) $R^2 = 0.34$, $P < 0.0001$ and (b) relationship of N₂O flux to photosynthetically active radiation (PAR) from OSR under NO₃ addition (closed triangles, short dashed line), $R^2 = 0.35$, $P < 0.0001$. Relationship of the hourly mean ($n = 5$) normalized NEP (c) and N₂O (d) to PAR, averaged over the study period for three nitrogen treatments with a second order polynomial function fitted: NEP- FER – closed circles, solid line, $R^2 = 0.98$, $P < 0.0001$; NH₄ – open circles, long dashes, $R^2 = 0.98$, $P < 0.0001$; NO₃ – closed triangles, short dashes, $R^2 = 0.98$, $P < 0.0001$. N₂O- FER – closed circles, solid line, $R^2 = 0.79$, $P < 0.0001$; NH₄ – open circles, long dashes, $R^2 = 0.62$, $P < 0.0001$; NO₃ – closed triangles, short dashes, $R^2 = 0.71$, $P < 0.0001$.

a transpiration-mediated flux of ca. 10 000 μg N₂O m⁻² h⁻¹ is theoretically possible, an ancillary experiment conducted during this study (data not shown) using short-term shading of the OSR vegetation to induce stomatal closure revealed no difference between fluxes of N₂O from shaded and unshaded vegetation, suggesting this was not a significant contributing factor.

We found strong evidence to suggest that PAR, rather than soil temperature, drove diurnal N₂O flux variation. Christensen (1983) suggested that PAR influenced N₂O flux and Das *et al.* (2012) specifically investigated its role on N₂O flux, but concluded its influence was limited to warming the soil. In our study, the relationship strengthened with increasing applied proportion of NO₃-N, the substrate for denitrification. As C availability drives denitrification both directly (Firestone & Davidson, 1989) and indirectly as increased microbial respiration depletes O₂ (Farquharson & Baldock, 2008), it is logical that by mediating exuded photosynthate PAR strongly influences N₂O emission when vegetation

is present. In a mesocosm experiment measuring GHG fluxes from bare agricultural soil, Ineson *et al.* (unpublished data) unequivocally demonstrated that without labile C, N₂O fluxes were negligible even under high rates of mineral N addition. However, we have not found any explanatory models of measured N₂O fluxes which use PAR, whilst soil organic carbon (SOC) or dissolved organic carbon (DOC) has only occasionally been used to explain N₂O fluxes from soils (e.g. Ambus & Christensen, 1993; Kaiser *et al.*, 1996; Lemke *et al.*, 1998; Harrison & Matson, 2003; Petersen *et al.*, 2008).

N₂O fluxes are notoriously difficult to model, especially at fine temporal resolution (Fitton *et al.*, 2014b), and although the models, DNDC (Abdalla *et al.*, 2009), DailyDayCent (Fitton *et al.*, 2014a) and ECOSSE (Dondini *et al.*, 2016), include various estimates of SOC, they also do not use PAR as a driving input. Furthermore, model validation often uses intermittent, daily flux measurements (e.g. Von Arnold *et al.*, 2005; Perdomo *et al.*, 2009; Johnson *et al.*, 2010; Gauder *et al.*, 2012; Jeuffroy

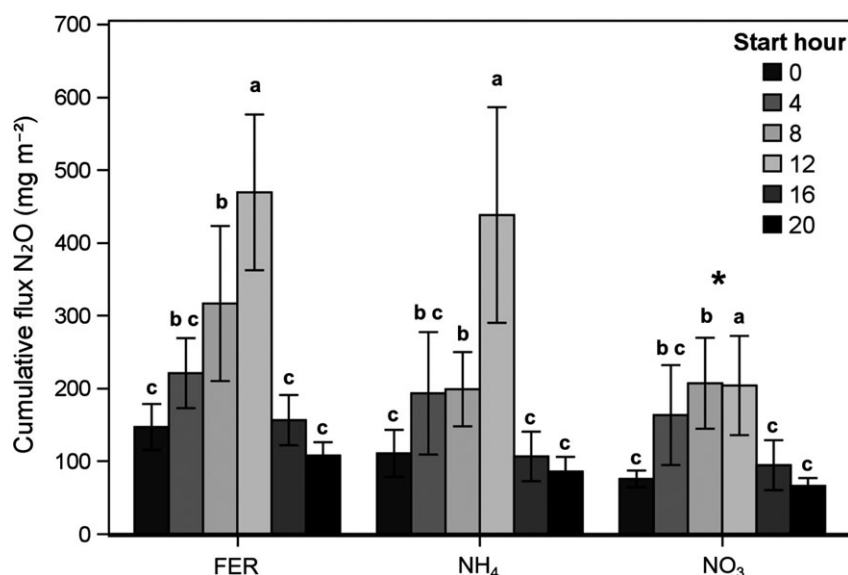


Fig. 8 The effect of sampling time on the estimate of mean (± 1 SE) cumulative flux of N₂O from OSR under three different nitrogen treatments. Significant ($P < 0.001$) differences in sampling time are shown (two-way ANOVA testing for effect of treatment and sampling time), times with similar letters do not differ (Duncan multiple range *post hoc* test). Time bins are: 0- 00–03:59 4- 04–07:59 8- 08–11:59 12- 12–15:59 16- 16–19:59 20- 20–23:59. Cumulative flux of N₂O was significantly lower ($P < 0.04$) from NO₃ than from FER treatment (*).

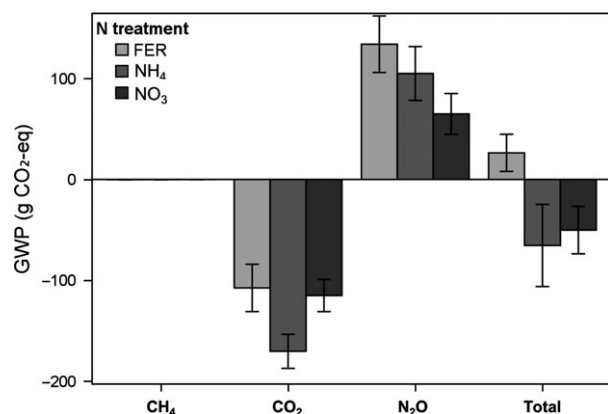


Fig. 9 Mean ± 1 SE total fluxes of CO₂, N₂O and CH₄ from oil-seed rape under three different nitrogen regimes, $n = 5$ shown in terms of global warming potential (GWP) as calculated over a 100 year period (Myrhe *et al.*, 2013) and is expressed in terms of CO₂-equivalents. Negative values indicate net uptake from the atmosphere and positive values net emission. OSR, oilseed rape.

et al., 2013), which rarely acknowledge the importance of selecting the appropriate time of day for sampling, despite this being essential to accurate GHG budgeting (Keane & Ineson, 2017). The interdiel and diel flux variation reported here underlines how systematic errors may occur when subdaily measurements are used to extrapolate long-term cumulative fluxes.

The diurnal variation in N₂O fluxes here was clearly linked to PAR, but PAR (NO₃ treatment) and soil

temperature (FER and NH₄) were important drivers over the entire study. We suggest that most N₂O was produced by denitrification, thus driven by organic C in NO₃ collars, but denitrification in the FER and NH₄ treatments was partly coupled to nitrification hence the association with soil temperature (Fig. 7(a)). It is noteworthy that there was no significant relationship between N₂O fluxes and soil moisture, which is often cited as one of the key drivers of N₂O production (Skiba *et al.*, 1998; Skiba & Smith, 2000; Dobbie & Smith, 2003b). A possible explanation is that soil moisture, ranging between 50 and 75% water-filled pore space (WFPS) throughout the study, was variously favourable to both nitrification and denitrification, processes which have different WFPS optima (Bateman & Baggs, 2005).

The pronounced variation in N₂O fluxes presented here was captured due to the high temporal resolution of SkyLine2D. The automated system measured CO₂, CH₄ and N₂O from OSR for 21 days, providing nearly 4000 flux measurements and the clear chamber ensured that fluxes included sinks and sources from soil and vegetation. Such data from tall vegetation are rare without using eddy covariance (EC) equipment, which currently cannot measure at the spatial resolution required to test hypotheses in replicated, manipulation experiments. Furthermore, SkyLine2D overcomes the shortcomings of previously described automated systems, such as low ($n < 10$) replication (e.g. Breuer *et al.*, 2000; Nishimura *et al.*, 2005; Barton *et al.*, 2008; Morris *et al.*, 2013), long

chamber closures (e.g. Breuer *et al.*, 2000: 45–60 minutes) or storage of samples for subsequent laboratory analysis (Ambus *et al.*, 2010; Juszczak & Augustin, 2013).

The high N₂O emissions across all treatments, even at 50% of the management applied N rate, demonstrate how important this gas is for crop GHG balance. Nitrogen uptake efficiency is a problem in OSR, where it is as low as 50% (Bouchet *et al.*, 2016) and our findings underline this inefficiency. We suggest that as the management fertilizer rate, which received double the N of the NH₄ treatment, either increased crop biomass, N content (unpublished data) or N₂O emissions that fertilizer is lost through immobilization or leaching, as outlined in Bouchet *et al.* (2016). We have shown that PAR, probably by supplying labile C to facilitate denitrification, is a strong driver of N₂O emissions, and its inclusion in GHG flux models should improve model accuracy, a vital tool to mitigate climate change. We would like to see work carried to manipulate diurnal fluctuation in DOC to directly investigate its effect on N₂O fluxes. Additionally, the pronounced diurnal pattern in N₂O flux demonstrated here underlines the critical importance of high-frequency, high spatial resolution measurements. If automation is not possible, based on our data, the appropriate sampling for OSR at this site would be around 08:00 or 16:00, to coincide with the daily mean flux. However, as diurnal patterns of N₂O flux differ between locations and crops (Alves *et al.*, 2012), we stress the importance of characterizing any diurnal pattern before selecting the appropriate sampling time, if single daily measurements are to be used in flux studies. Finally, the large GHG emission from the OSR suggests there are more suitable feedstocks which should be used for biofuel production.

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